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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 773688681 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Hur	Koser	Branford, Connecticut

☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

Microfabricated Cellular Traps Based on Three-Dimensional Micro-Scale Flow Geometries

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

- [x] Specification *Number of Pages* 16
- ☐ Drawing(s) *Number of Sheets*
- ☐ Application Data Sheet. See 37 CFR 1.76
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Respectfully submitted,

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Date 02/17/04

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DISCLOSURE DATE: January 21, 2004 Yale OCR No. 1641

TITLE
Microfabricated cellular traps based on three-dimensional micro-scale flow geometries

INVENTOR
Hur Koser, Electrical Engineering

BACKGROUND

Chemotaxis is a fundamental cellular process that describes the motile response of cells to the presence of a concentration gradient of a given chemical in solution. Until very recently, fundamental shortcomings in experimental apparatus allowed the determination of the presence or absence of a chemotactile response, while precluding its exact quantification. Efficient, highly controllable chemical gradient pattern generation chambers fabricated using soft lithography techniques offers an attractive alternative to existing macro-scale devices. While such microfluidic devices have recently been demonstrated [1], their entire potential as efficient drug discovery platforms has not been exploited yet. This is partly because the chemical gradient within the microfluidic device can only be maintained with continuous flow, and most cells require special substrate surface chemistry to maintain their positions within the field of view of a microscope objective. The process of chemically modifying the substrate surface for a particular cell type is complex, may be costly and difficult. Even then, the shear force of the flow over the membrane of the cell is disruptive. In the case of the original reference [1], rabbit neutrophils that stuck to the glass substrate were used.

Not all cell types, however, move in an amoeba-like fashion like neutrophils. Bacteria, for instance, move via a set of propelling organs, such as a flagella or cilia. A bacterium responds to chemical gradients of chemo-attractants (or chemo-repellents) by performing a biased random walk up (or down) the gradient, with short periods of straight swimming interrupted by tumbling that reorients the cell preferentially towards the gradient. For that reason, surface chemistry modification alone will not result in microfluidic chemotaxis chambers compatible with bacteria. The flow regimes that are practically achievable for sustaining chemotactic gradients in fluidic channels are on the order of a micron per second or faster (a mm/s is about the norm); within the resolutions needed to observe bacteria under the microscope, the cells simply flow out of the field of view in seconds. What is needed to observe and quantify chemotaxis in bacteria effectively is a means of exposing them to the concentration gradient of the solute of interest while keeping them long enough under the field of view.

DESCRIPTION

Existing methods of cell trapping and manipulation within microfluidic devices, such as dielectropherosis [2], exert the cells to spatially non-uniform external forces that will prove disruptive in efforts to measure the natural motile response of the cells. The ideal chamber that is suited to all types of cells, including bacteria, would involve some way of essentially freezing the chemotactic gradient locally, so that the cells are free to move and respond naturally to the chemotactic agent.

We propose to create a microfluidic chemotaxis chamber that addresses the issues raised above and accommodate fast, efficient, repeatable, parallel measurements on virtually any motile cell. The proposed concept involves a microfluidic device with three distinct, coupled functional regions. The first compartment is positioned just after the fluidic inputs to the device, and is involved in creating the chemical gradient. This section could be based on the method illustrated in [1], or it could simply incorporate multiple input ports, each carrying the necessary concentration of the chemotactic agent. The main flow channel that follows is wide enough to ensure that diffusion across its width is relatively slow and, given the flow speed, the concentration gradient is essentially maintained throughout its length. The final set of compartments is positioned underneath the main flow chamber, lying as perpendicular trenches across its width . It is the incorporation of these trenches within the fluidic channels that constitute the invention disclosed herein.

Flow within the trench regions are generally orders of magnitude slower than (essentially stagnant compared to) the flow in the main channel above them. The dimensions are easily customizable to make sure that the cells inside the trenches can move faster than the flow around them. As long as the depth of the trenches are much smaller than the width of the main flow channel, the concentration gradient in the flow channel diffuses down and is established along the length of the trenches as well. Therefore, the cells within the confinement trenches are exposed to the desired chemotactic gradient, without appreciable disruptive shear forces over their membranes.

This simple approach is effective for observing chemotaxis on relatively large, slow moving cells, such as lymphocytes. For smaller, faster moving organisms, the trenches may be capped on top with a thin layer of SU-8, with periodic thin slots ("jail bars") that allow the concentration gradient to diffuse through, while preventing the trapped cells from escaping.

This invention encompasses the integration of micro-trenches within microfluidic chambers for cellular trapping, manipulation and real-time analysis of biological phenomena, including chemotaxis. The invention captures the design methodology for these traps, as well as the fabrication means and the associated external control mechanism that allows rapid, reversible and fully controlled changes in the chemical environment to which trapped cells are exposed.

NOVELTY

Microfluidic channels are mainly two-dimensional arrangements of small fluidic compartments. Structures in the third dimension (out of the plane of the substrate) are routinely constructed as well, though they are rarely designed to affect the flow profile in the third dimension. The main novelty of this technology is the incorporation of cell trapping trenches that use the formation of local eddy-current flows to create calm, cell-sustaining regions just underneath a relatively fast moving flow region. The main advantage of this approach is that the fast moving flow region can support a stable, controlled chemical gradient just over the trench, while exerting virtually no disturbing forces on the trapped cells. The chemical gradient and nutrients present in the main flow channel diffuse down into the trenches very effectively when the dimensions of the trench are small compared to those of the main flow channel. This approach eliminates any need for expensive and time-consuming surface chemistry adjustments that would otherwise be required for each different species of cells. In this way, multiple trenches can be formed on the same substrate, and multiple cell species can be supported simultaneously. In the context of miniature organs mentioned earlier, this capability to work with multiple cell species simultaneously opens the door to the creation of "physiological system" models, where a simplified version of a multicellular organism could be simulated on such devices.

To the best of this inventor's knowledge, there exists no other configuration or design with the capabilities and advantages listed above. Although chemotaxis gradient pattern generators have been demonstrated before [1], effective, completely non-intrusive cell trapping mechanisms to integrate with them have not.

USES

- These microfluidic chambers and associated support hardware will prove invaluable in determining an accurate model for the exact mechanism of chemotaxis in virtually all cell types and species. They would also provide a foundation for understanding complex hormonal and chemical signaling pathways. It is expected that these devices will generate a wealth of new information and knowledge in medical areas ranging from pregnancy to sexual disfunction, from aging to depression.

- The main use of these devices will be in drug discovery and pharmacological studies. By studying competing chemotactic agents simultaneously on multiple cell species on the same device (where multiple trenches will be filled with different cell types), drug impact studies can be conducted and the potency of new drug candidates can be tested directly on a cellular level. Potential applications would include drug testing for immunological disorders, HIV, Anthrax and various forms of cancer.

- These chambers would allow researchers to conduct side-effect testing of drugs simultaneously with potency measurements.

- The idea of the trenches could be generalized to create a network of such geometries integrated within microfluidic channels. Such configurations would allow the creation of complex "societies of cells", even miniature organs, within these devices. The many uses of such devices would be mainly in the biomedical engineering research and health industry.

- It is this inventor's intention to use these microfabricated devices in conjunction with neurotrophic growth factors in order to create controlled neural networks on substrates. Hippocampal neurons from rats and mice could be placed in the aforementioned trenches and their axon growth could be directed using specific concentration gradient patterns of the growth factors. Such networks could allow scientists to study the effects of various drugs and/or stimulants on the efficacy of synaptic transmissions. Studies on such debilitating conditions as Alzheimer's and Parkinson's disease can be conducted in much more controlled and easy-to-observe conditions.

RESEARCH STILL NEEDED

One potential challenge lies in determining the best mechanism to introduce cells into the trenches. Side input ports will be used to directly connect to the trench structures, and cells may be introduced by a pressure driven system or a syringe pump. Keeping a particular cell inside a given trench will initially prove difficult, as the cell may either swim up into the main flow channel (and get caught in the stream to be swept out of the device) or simply swim out of the trench into the side injection ports. The former challenge may be easily addressed by the "jail bar" structure, in which rectangular slot openings narrower than the cell dimensions are integrated within a roof over the trench to allow diffusion to take place while physically confining the cells. Preventing the cells from swimming out of the trench on the side is trickier; it might require integrated fluidic valves or additional flow streams along the edges of the main flow channel carrying a chemotactic repellent. Further research is needed to address this latter issue.

ADDITIONAL COMMENTS

This invention is rather simple in its basics and yet quite fundamental in developing the ability to manipulate cells chemically in microfluidic environments. As mentioned in the Description section, creating chemotaxis gradients is not the novel part of this invention; it is the cellular trapping mechanism, its fabrication process and the associated external hardware that make up a coherent and very useful invention.

LAB RECORDS

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I. BACKGROUND

i) MEMS and Microfluidics

Over the recent decades, technology originally developed to bulk produce semiconductor devices such as microprocessors has been successfully applied in the manufacture of miniature mechanical sensors and actuators that are known today as micro-electro-mechanical systems (MEMS) [1, 2]. Some MEMS devices, such as accelerometer chips in car airbag inflation systems, miniature pressure sensors, gyroscopes, and ultra bright display chips have already revolutionized their respective markets [3, 4, 5, 6]. Lately, MEMS fabrication techniques have been applied towards the creation of fluidic devices with micron-scale features. These microfluidic devices offer the potential to integrate many different aspects of a chemical or bioengineering laboratory onto a single chip (thereby functioning as a "lab-on-a-chip") that is able to handle miniscule amounts of sample and produce highly accurate and speedy results [7]. Thanks to a stamping and molding process involving poly-dimethylsiloxane (PDMS), the prototyping process for microfluidic devices is rather simple, rapid and cheap [8, 9].

Microfluidic devices made out of PDMS are robust, easy-to-handle, and most importantly, disposable. Currently, they are a popular medium of choice for lab-on-a-chip research. High levels of integration, including hundreds of control valves, separation chambers have already been demonstrated in PDMS devices [10]. These devices are not only convenient in research, but they also lend themselves naturally to the creation of a design-based, hands-on bioengineering teaching laboratory.

ii) Chemotaxis

Chemotaxis is a fundamental cellular process that describes the motile response of cells to the presence of a concentration gradient of a given chemical in solution. We are mainly interested in the creation of efficient, highly controllable chemical gradient pattern generation chambers fabricated using soft lithography techniques, in order to *quantify* chemotaxis *in virtually any cell.* Until very recently, fundamental shortcomings in experimental apparatus allowed the determination of the presence or absence of a chemotactile response in a population of cells, while precluding its exact quantification on the level of an individual cell. Efficient, highly controllable chemical gradient pattern generation chambers fabricated using soft lithography techniques offer attractive alternatives to existing macro-scale devices [11, 12]. These devices utilize laminar flow and rapid diffusion that are characteristic in micro-scale flow channels to create spatially and temporally stable chemical concentration patterns.

The shortcoming of these microfluidic devices is that the chemical gradient within the microfluidic device can only be maintained with continuous flow, and most cells require special substrate surface chemistry to maintain their positions within the field of view of a microscope objective. The process of chemically modifying the substrate surface for a particular cell type is complex, may be costly and difficult. Even then, the shear force of

the flow over the membrane of the cell is disruptive. In the case of the original reference [12], rabbit neutrophils that stuck to the glass substrate were used.

Not all cell types, however, move in an amoeba-like fashion like neutrophils. Bacteria, for instance, move via a set of propelling organs, such as a flagella or cilia. A bacterium responds to chemical gradients of chemo-attractants (or chemo-repellents) by performing a biased random walk up (or down) the gradient, with short periods of straight swimming interrupted by tumbling that reorients the cell preferentially towards the gradient. For that reason, surface chemistry modification alone will not result in microfluidic chemotaxis chambers compatible with bacteria. The flow regimes that are practically achievable for sustaining chemotactic gradients in fluidic channels are on the order of a micron per second or faster (a mm/s is about the norm); within the resolutions needed to observe bacteria under the microscope, the cells simply flow out of the field of view in seconds. What is needed to observe and quantify chemotaxis in bacteria effectively is a means of exposing them to the concentration gradient of the solute of interest while keeping them long enough under the field of view.

Existing methods of cell trapping and manipulation within microfluidic devices, such as dielectropherosis [13], exert the cells to spatially non-uniform external forces that will prove disruptive in efforts to measure the natural motile response of the cells. The ideal chemotaxis chamber that is suited to all types of cells, including bacteria, would involve some way of essentially freezing the chemotactic gradient locally, so that the cells are free to move and respond naturally to the chemotactic agent.

We propose to design, fabricate and test a microfluidic chemotaxis chamber that addresses the issues raised above and accommodate fast, efficient, repeatable, parallel measurements on virtually any motile cell. The resulting chambers will then house the cellular signaling and programming nanotools that are described above, as well as the integrated electrical signaling pathways that may be necessary. Hence, the microfluidic chambers will allow the direct manipulation of individual cells through an ability to precisely control the chemical environment that the cells occupy.

II. DESIGN

The general architecture of the proposed microfluidic devices is illustrated in Fig. 1. The geometric details and dimensions of the micro-channels are variable, depending on the particular cell size and type, as well as the specific chemical environment that is desired. A fluid dispensing system, including miniature electronically-controlled valves, precede the input ports that carry the desired chemical solutions to the main flow channel[1]. Such a system allows the user to control flow velocities accurately and to change flow compositions rapidly. A separate system controls the side inlets through which the

[1] Such a system can be built in-house using a simple pressure-based driving mechanism and miniature solenoid valves. There are also commercially available and highly accurate micro-dispensing systems, such as IVEK Corporation's Multiplex system (http://www.ivek.com).

bacteria or other cells of interest are introduced into "trapping trenches" just underneath the main channel.

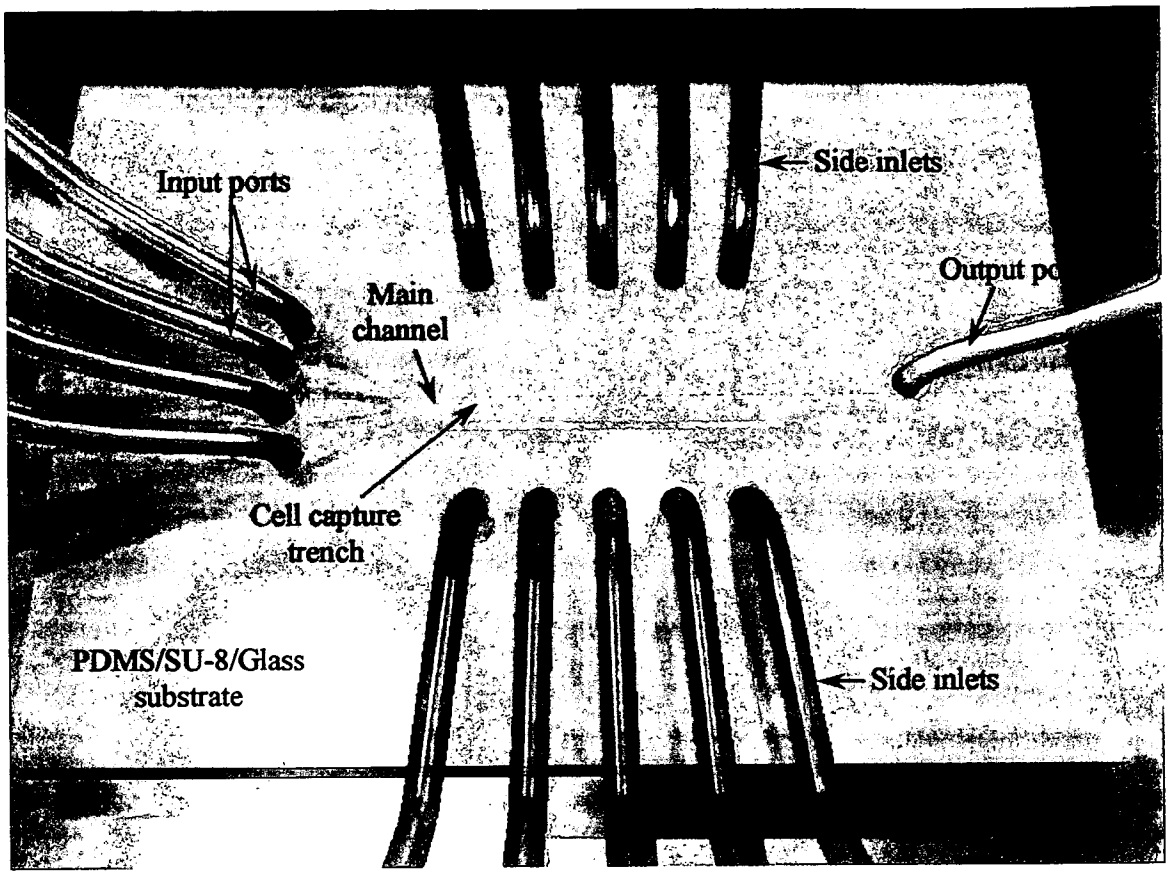

Fig. 1: The microfluidic device is formed from a stack of glass, SU-8 and PDMS (the resulting device is transparent and compatible with optical microscopy). The main flow channel is used to create the desired chemical environment, while the side inlets introduce solutions containing individual cells into specialized containment trenches.

The microfluidic device itself is composed of three distinct, coupled functional regions. The first compartment is positioned just after the fluidic inputs to the device, and is involved in creating the chemical gradient. This region's geometry may be as simple as multiple input channels merging to form the main channel (essentially, what is depicted in Fig. 1), or as complicated as in [12], where a small number of input ports are used to create sophisticated chemical concentration gradients (Fig. 2). The common underlying mechanism involves laminar flow and very rapid diffusion across micro-scale dimensions, in order to achieve controlled mixing of the contents of the input ports.

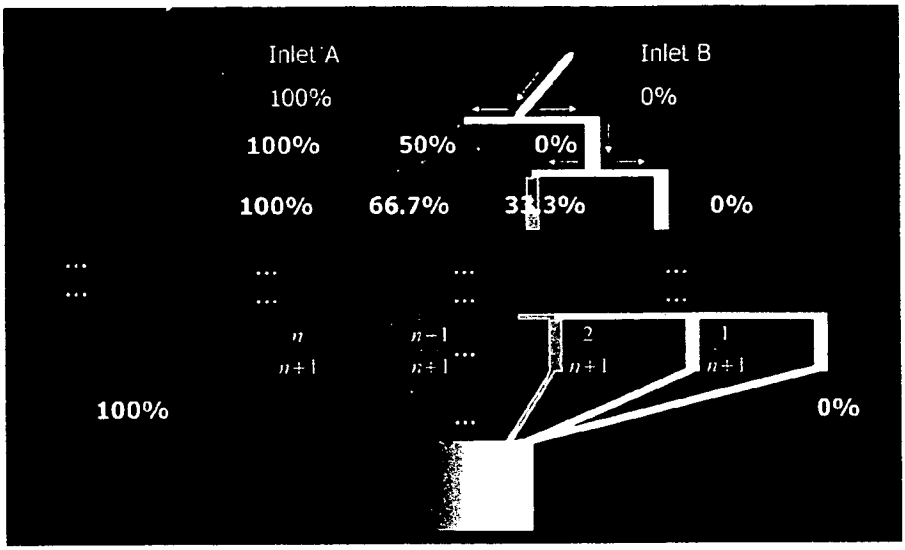

Fig. 2: The schematic description of a sample linear gradient pattern generator. At each level, the length of the branches is chosen long enough to ensure complete mixing.

The main flow channel that follows is wide enough to ensure that diffusion across its width is relatively slow and, given the flow speed, the concentration profile achieved at the input section is essentially maintained throughout its length. The final set of compartments is positioned underneath the main flow chamber, lying as perpendicular trenches across its width; these are the containment trenches that are designed to sustain the individual cells (Fig. 3).

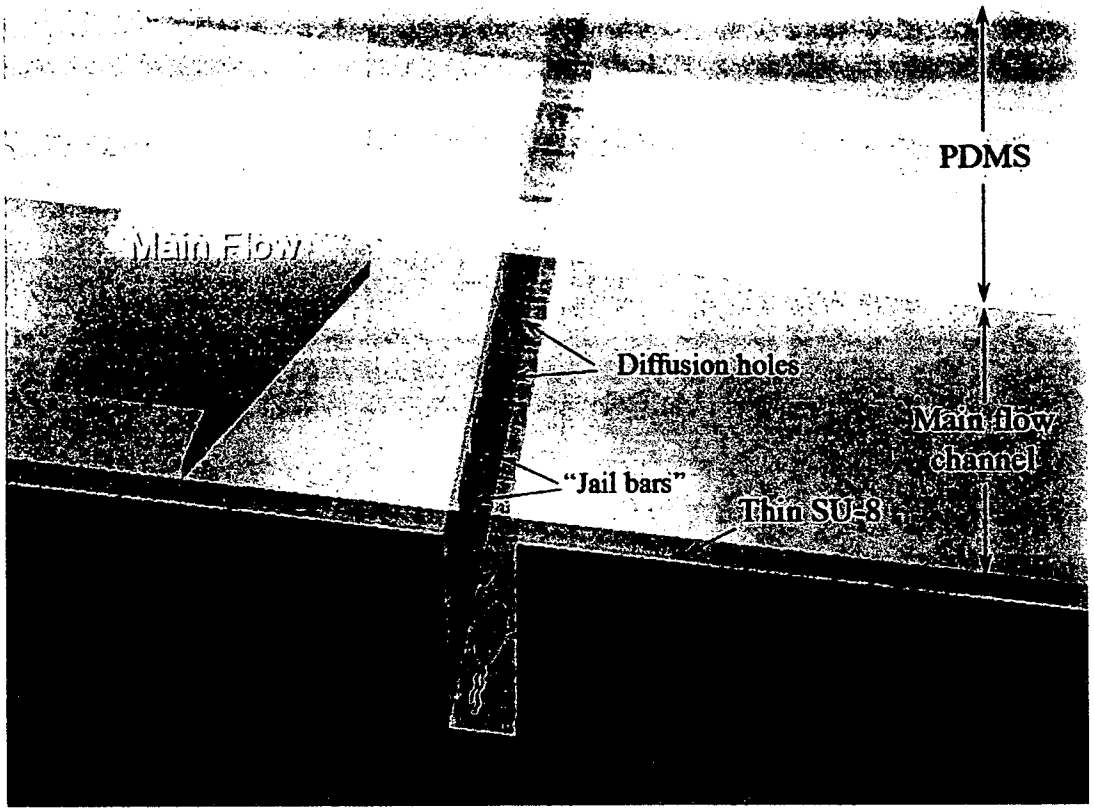

Fig. 3: A cut-away view of the microfluidic device depicting a cell containment trench. Note that for illustration purposes, vertical dimensions and the size of the diffusion holes are exaggerated. Thanks to rapid diffusion across the smaller two dimensions of the trench, the main flow continuously replenishes and controls the chemical environment under the "jail bars".

The basic purpose of the trenches underneath the main flow channel in Fig. 3 is to provide a mechanism to confine cells which are initially introduced through the side inlets. Since the Reynolds' numbers associated with the flow are very small (even for the maximum practically achievable flow rates), the main flow stays laminar over the trench geometry. However, extensive simulations of Navier-Stokes equations have shown that appropriately chosen trench dimensions (the width and the height) result in the formation of a local eddy within the confinement trench. It has been confirmed that these local eddies tend to form virtually at any operating flow rate given suitable trench dimensions. What is more, the average flow rate within the trench is then orders of magnitude slower than the flow in the main channel.

Fig. 4 illustrates the flow profile just over and inside a 50um x 50 um trench, covered on top by a 20 um opening. The average flow rate within the main channel is 1 mm/s, whereas the average flow speed inside the trench is just a few microns per second. Introduction of multiple, smaller openings on top across a given trench cross-section reduces the flow rate inside the trench to less than 1 um/s (Fig. 5). This geometry then allows experiments in which cells can swim virtually freely along the length of the trench without experiencing any appreciable drag or shear forces from the flow. Using standard MEMS fabrication techniques, the trench holes can easily be chosen to be smaller than

the dimensions of the trapped cells, so as to guarantee that the cells will not accidentally swim out of the confinement trenches.

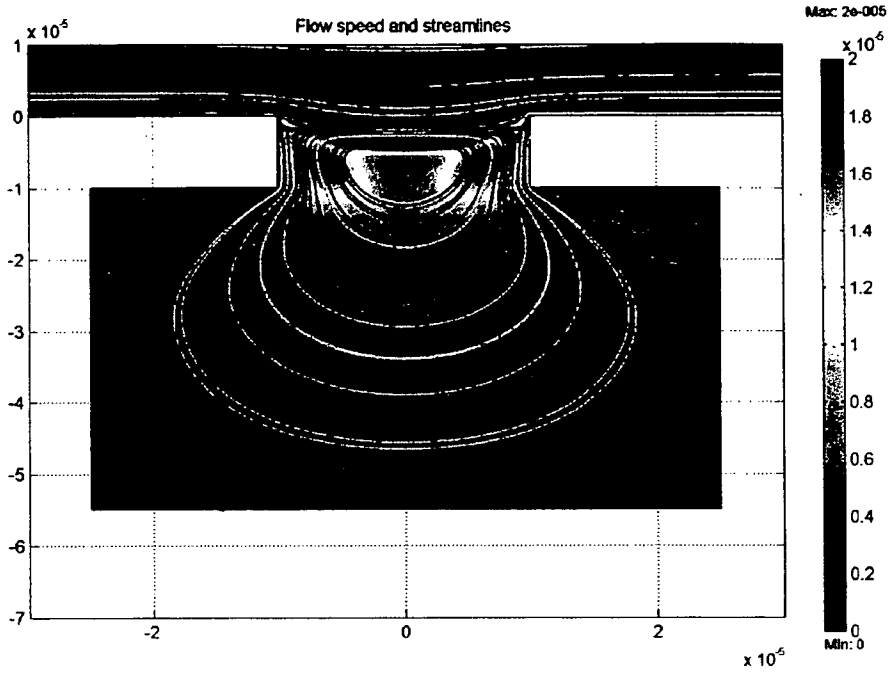

Fig. 4: A numerical simulation result overlaid over a schematic side view of a cell confinement trench underneath the main flow channel. The average flow rate within the trench is just a few microns per second.

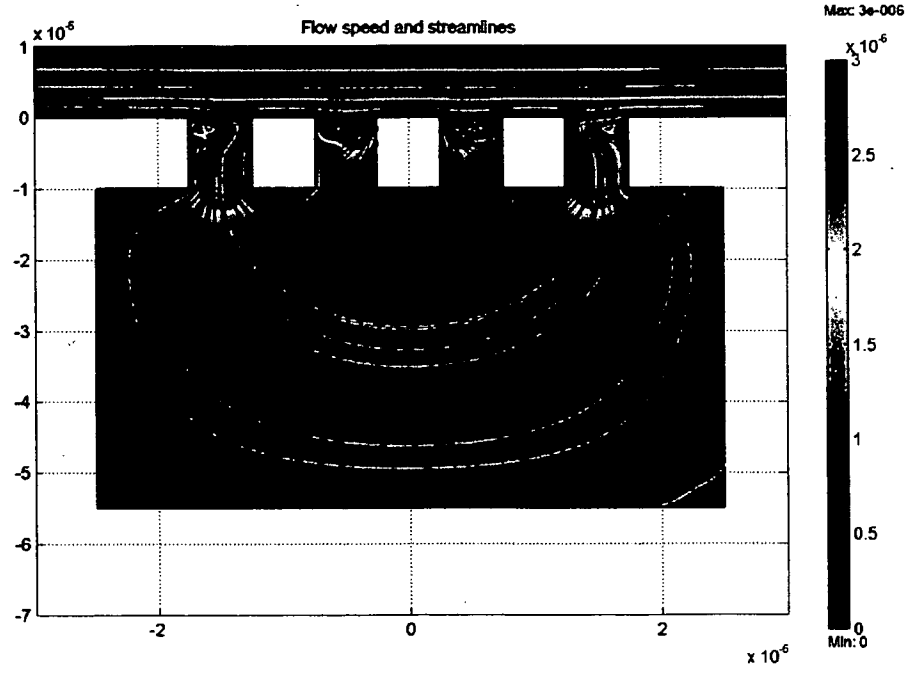

Fig. 5: Using smaller, multiple diffusion holes across the cross-section reduces the average flow rate within the trench to under 1 um/s.

The other advantage of this geometry is that the gradient pattern established in the main flow is preserved spatially within the trench. This is because both the nutrients and the chemotactic concentration gradient within the main flow above continuously diffuse through the diffusion holes. As long as the depth of the trenches are much smaller than the width of the main flow channel, this diffusion is complete. Therefore, the cells within the confinement trenches can be exposed to the desired chemotactic gradient.

III. FABRICATION

The fabrication of the microfluidic devices are based on the already established soft lithography techniques [9]. Fig. 6 illustrates the basic steps of the microfabrication process. Essentially, glass microscope slides will be used as substrates, and photosensitive polymers will help define the channel geometries. Any necessary general surface treatments can be performed after the fabrication steps shown in Fig. 6, and prior to bonding PDMS to the confinement trench substrate.

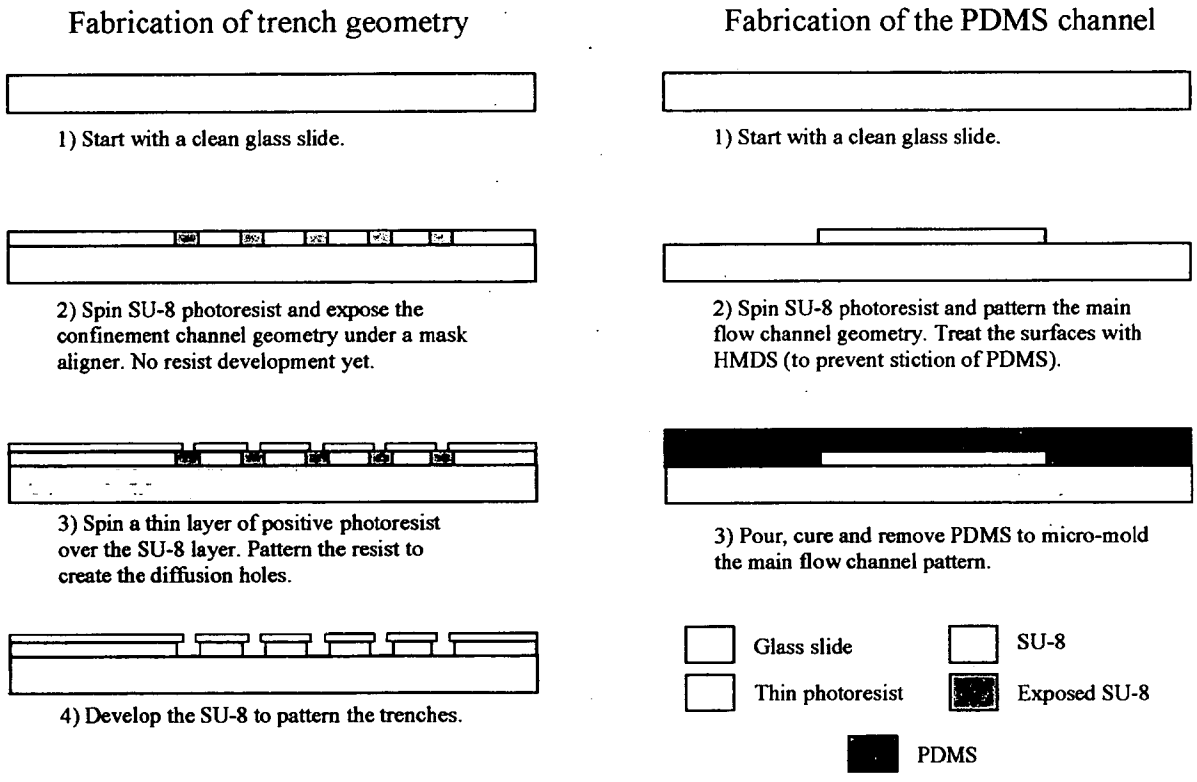

Fig. 6: Fabrication process flows for both the trench geometry and the PDMS main flow channel components.

IV. IMPACT AND PROPOSED STUDIES

With this application of microfluidics technology, multiple trenches can be formed on the same substrate, and multiple cell species can be supported simultaneously. The capability to work with different cell species on the same device opens the door to the creation of "physiological system" models, where a simplified version of a multicellular organism could be simulated. When the cells of interest are bacteria, the effects of chemicals, new drugs or antibacterial agents can be studied simultaneously on different subspecies. Real-time observation of chemotaxis behavior on multiple cell types allows these devices to be used as drug discovery platforms.

Chemotaxis itself can be used as a cellular manipulation scheme to coax the cells to specific locations within the trenches where interactive sensors are located. In this respect, these chemotaxis chambers are ideal candidates for housing the nano-scale sensing and signaling posts that form the basis of this proposal. For instance, if the posts are located at the center of a confinement trench, then a chemotactic attractant can be used to create a concentration profile with a peak at the center of the main flow channel. In this manner, the cells would be drawn to the center of the trench and eventually some would make contact with the posts.

We recognize that chemotaxis is not the only means to manipulate cells, though it is probably the most "natural" means to do so. After all, motile cells in their natural habitats continuously respond to gradients of chemicals that correspond to specific signals, such as those from other cells, or the presence of food. Nevertheless, the MEMS approach easily accommodates the integration of other cellular manipulation schemes, such as dielectropherosis traps or integrated micro-valves and pumps, into the chemotaxis chambers. When used in conjuction with a suitable chemotaxis profile, such methods will likely prove very effective in localizing individual cells in the vicinity of the integrated nano-posts.

One appealing aspect of the overall fluidic system is its capability to change on the fly the chemical environment that the cells are subjected to. Hence, once a given cell or bacterium connects with a nano-post structure, an amazing wealth of biologically relevant studies can be conducted.

V. RESEARCH CHALLENGES

One potential challenge lies in determining the best mechanism to introduce cells into the trenches. Side input ports will be used to directly connect to the trench structures and cells may be introduced by a pressure driven system or a syringe pump. Keeping a particular cell inside a given trench will initially prove difficult, as the cell may simply swim out of the trench into the side injection ports. This potential problem might require integrated fluidic valves or additional flow streams along the edges of the main flow channel carrying a chemotactic repellent. Intentionally introducing tiny air bubbles on the

side boundaries of the trenches may also help trap the cells. This is one of the first challenges that will be addressed with different design schemes and experiments.

VI. FACILITIES AND SUITABILITY

Yale suitability: Yale University is uniquely suitable for providing the required facilities, the interdisciplinary work and collaborations associated with this proposal. The university boasts an excellent medical school, which is teeming with expertise in health sciences and experience with cell cultures. We also have a world renowned Biology Department, and a newly formed Biomedical Engineering Department. A brand-new building dedicated to Biomedical Engineering is under construction. The Electrical Engineering Department is in possession of a 2600 sq. ft., class 100 cleanroom space fully capable of supporting the production of microfluidic devices, and a friendly and capable staff supporting it. We have just purchased a brand-new EVG620 mask aligner to advance our wafer handling capability up to 6''. Prof. Koser's bioMEMS lab has been designed to be both a cleanroom and a BioSafety Level II laboratory. It features two full size chemical hoods, one of which will be dedicated to PDMS work, a biosafety cabinet, an incubator, fluorescence microscopes, class 1000-10000 cleanroom conditions and all the working space necessary to support many graduate students simultaneously. For all those reasons, as well as the commitment of our department and much interdisciplinary collaboration already established, we believe the timing is just right for this project to get off to a good, successful start.

PI suitability: The PI holds B.S. degrees in Electrical Engineering (EE) and Physics, and Master of Engineering and Ph.D. degrees in EE (MEMS). He has extensive experience in MEMS design, analysis, fabrication, testing, instrumentation, software creation, animation and simulation development. He has helped teach and mentored student design teams in a MEMS design class. He conducted a six month long Post Doc at MIT designing, fabricating and testing microfluidic chambers for use in a classroom setting. He has already created the laboratory tools and the analysis software needed for this study. Prof. Koser is the recipient of the Information Technology Services Innovation Award, as well as the Moore Fund Award at Yale University. The funds from these awards are being used to develop an online tutorial-based teaching module that is intended to provide BME students a hands-on learning opportunity about micro-scale diffusion, transport and microfluidics.

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Microfabricated Cellular Traps Based on Three-Dimensional Micro-Scale Flow Geometries (Yale OCR 1641)

Inventor: Hur Koser

We Claim:

1. A method for the design, fabrication, and integration of micro-trenches within microfluidic chambers to create a cellular trapping mechanism, and to manipulate and perform real-time analysis of biological phenomena, including, but not limited to, chemotaxis.